Figs. 2A-2D give the amino acid sequence comparison of the KS domains and the CLF domains of representative Type II PKS gene clusters. The active site Cysteine (C) of the KS domains is arrowed in the Figure and aligns with the Glutamine (Q) or glutamic acid (E) of the CLF domains. The abbreviations used, and the relevant Genbank/EMBL accession numbers are: GRA: granaticin from *Streptomyces violaceoruber* (X63449); HIR: unknown polyketide from *Saccharopolyspora hirsuta* (M98258); ACT, actinorhodin from *Streptomyces coelicolor* (X63449); CIN: unknown polyketide from *Streptomyces cinnamonensis* (Z11511); VNZ: jadomycin from *Streptomyces venezuelae* (L33245); NOG: anthracyclines from *Streptomyces nogalater* (Z48262); TCM: tetracenomycin from *S. glaucescens* (M80674); DAU: daunomycin from *Streptomyces sp.* C5 (L34880); PEU, doxorubicin from *Streptomyces peucetius* (L35560); WHI: WhiE spore pigment from *Streptomyces coelicolor* (X55942).

Fig 3 shows the gene organisation of the PKSs for three 16-membered ring macrolides, tylosin, spiramycin and-niddamycin.

Figs. 4A-4C show the amino acid sequence alignment of KSq-ATq loading didomains of the PKSs for niddamycin, platenolide(spiramycin), monensin, oleandomycin and tylosin. The sequences for the monensin and oleandomycin loading didomains have not been previously disclosed.

Fig. 5 The enyzmatic steps that convert 6-deoxyerythronolide B into erythromycin A in *Saccharopolyspora erythraea*.

Fig. 6 is a diagram showing the construction of plasmid pJLK117.

Fig. 7 shows the structures of two oligonucleotides.

Please replace the following paragraphs on pages 32, 33, 43, 47, 49, 53, 58, 59, 63, 64, 70 and 72 to add sequence identifiers, as follows:

(Paragraph at page 32, line 13 through page 33, line 10.) The following synthetic oligonucleotides: 5' - CCATATGGCCGCATCCGCGTCAGCGT- 3' (SEQ ID No. 28) and 5' - GGCTAGCGGGTCCTCGTCCGTGCCGAGGTCA- 3' (SEQ ID No. 29) are used to amplify the DNA encoding the putative monensin-producing loading module using a cosmid that contains the 5' end of the putative monensin-producing PKS genes from *S. cinnamonensis* or chromosomal DNA of S.*cinnamonensis* as template. The PCR product of 3.3 kbp is purified by gel electrophoresis, treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which has been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid pPFL40. Plasmid pPFL40 was identified by restriction pattern and sequence analysis.

(Paragraph at page 33, line 11 through page 33, line 2.) Plasmid pHD30His is a derivative of pNEWAVETE

(PCT/GB97/01810) which contains the avermectin loading module, erythromycin extension modules 1 and 2 and the ery thioesterase domain. Plasmid pNEWAVETE was cut with is EcoRI and HinDIII and a synthetic oligonucleotide linker was inserted that encodes the addition of a C-terminal polyhistidine tail to the polypeptide. The following oligonucleotides:

5' -AATTCACATCACCATCACCATCACTAGTAGGAGGTCTGGCCATCTAGA- 3' (SEQ ID No. 30) and

B.3 Cont

5' -AGCTTCTAGATGGCCAGACCTCCTACTAGTGATGGTGATGGTGATGTG- 3' (SEQ ID No. 31) were annealed together and the duplex was ligated to EcoRI-and HinDIII-cut pNEWAVETE. The resulting plasmid was cut with NdeI and XbaI and ligated into plasmid pCJR24 that had been previously cut with same two enzymes, to produce plasmid pND30His.

(Paragraph at page 43, line 24 through page 44, line 7.) The following synthetic oligonucleotides: 5' - CCATATGACCTCGAACACCGCTGCACAGAA- 3' (SEQ ID No. 54) and 5' - GGCTAGCGGCTCCTGGGCTTCGAAGCTCTTCT- 3' (SEQ ID No. 55) were used to amplify the DNA encoding the tylosin-producing loading module

B.4

using either cos6T (a cosmid that contains the tylosin-producing PKS genes from *S. fradiae*) or chromosoml DNA from *S. fradiae* as template. The PCR product of 3.3 kbp was purified by gel electrophoresis, treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The

ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid PpPFL39. Plasmid pPFL39 was identified by restriction and sequence analysis.

(Paragraph at page 47, lines 13-19.) A 411 bp DNA segment of the *ery*AI gene from *S.erythraea* extending from nucleotide 1279 to nucleotide is 1690 (Donadio, S. *et al.*, Science (1991) 2523:675-679) was amplified by PCR using the following synthetic oligonucleotide primers: 5' -TGGACCGCCGCCAATTGCCTAGGCGGGCCGAACCCGGCT- 3' (SEQ ID No. 32) and 5' -CCTGCAGGCCATCGCGACGACCGCGACCGGTTCGCC- 3' (SEQ ID No. 33).

(Paragraph at page 49, line 13 through page 50, line 5.) A DNA segment encoding the KSq domain from the *ole*AI gene of *S. antibioticus* extending from nucleotide 1671 to nucleotide 3385 was amplified by PCR using the following synthetic oligonucleotide primers: 5' -CCACATATGCATGTCCCCGGCGAGGAA- 3' (SEQ ID No. 34) and 5' -CCCTGTCCGGAGAAGAGGAAGGCGAGGCCG- 3' (SEQ ID No. 35) and chromosomal DNA from Streptomyces antibioticus as a template. The PCR product was treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid, pPFL31. The new *Nde* I site

bordering the insert is adjacent to the *Eco* RI site of the pUC18 polylinker while the new *Bsp* EI site borders the *Hin* dIII site of the linker region. Plasmid pPFL31 was identified by restriction and sequence analysis.

(Paragraph at page 53, line 18 through page 54, line 7.) The following synthetic oligonucleotides: 5' - CCATATGTCTGGAGAACTCGCGATTTCCCGCAGT- 3' (SEQ ID No. 36) and 5' - GGCTAGCGGGTCGTCGTCGTCCCGGCTG- 3' (SEQ ID No. 37) were used to amplify the DNA encoding the spiramycin producing loading module using chromosomal DNA from the spiramycin producer *S. ambofaciens* prepared according to the method described by Hopwood *et al.* (1985). The PCR product of 3.3 kbp was purified by gel electrophoresis, treated with T4 polynucleotide kinase and ligated to plasmid pUC18, which had been linearised by digestion with *Sma* I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual clones were checked for the desired plasmid pPFL41. Plasmid pPFL41 was identified by restriction patten and sequence analysis.

(Paragraph at page 58, line 18 through page 59, line 5.) The approximately 1.47 kbp DNA fragment of the eryAI gene of *S. erythraea* was amplified by PCR using as primers the synthetic oligonucleotides:

5' -TACCTAGGCCGGGCCGGACTGGTCGACCTGCCGGGTT- 3' (SEQ ID No. 38) and

5' -ATGTTAACCGGTCGCGCAGGCTCTCCGTCT- 3' (SEQ ID No. 39) and plasmid pNTEP2 (Oliynyk, M. *et al.*, Chemistry and Biology (1996) 3:833-839; W098/01546) as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with *Sma*I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK02 was identified by its restriction pattern and DNA sequencing.

(Paragraph at page 59, lines 8-20.) The approximately 1.12 kbp DNA fragment of the *ery*AI gene of *S. erythraea* was amplified by PCR using as primers the synthetic oligonucleotides: 5' -ATGTTAACGGGTCTGCCGCGTGCCGAGCGGAC- 3' (SEQ ID No. 40) and 5' -CTTCTAGACTATGAATTCCCTCCGCCCAGC- 3' (SEQ ID No. 41) and plasmid pNTEPH as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with *Sma*I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid, pJLK03 was identified by its restriction pattern and DNA sequencing.

(Paragraph at page 63, lines 12-26.) The approximately 2.2 kbp DNA segment of the rapB gene of *S. hygroscopicus* encoding

the reductive loop of module 10 was amplified by PCR using as primers the synthetic oligonucleotides: 5' -TAAGATCTTCCGACGTACGCGTTCCAGC- 3' (SEQ ID No. 42) and 5' -ATGCTAGCCACTGCGCCGACGAATCACCGGTGG- 3' (SEQ ID No. 43) and as template an approximately 7 kbp fragment, which has been obtained by digestion of cosmid cos 26 (Schwecke, T. *et al.* (1995) Proc. Natl. Acad. Sci. USA 92:7839-7843) with *Sca*I and *Sph*I. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with *Sma*l and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual colonies were checked for their plasmid content.

(Paragraph at page 64, line 17 through page 65, line 5.) The approximately 6.1 kbp DNA segment of the erythromycin PKS gene cluster of S. erythraea encoding the DNA fragment from the beginning of the ACP of module 2 to the beginning of the ACP of module 3 was amplified by PCR using as primers the synthetic oligonucleotides: 5' -TACCTGAGGGACCGGCTAGCGGGTCTGCCGCGTG- 3' (SEQ ID No. 44) and 5' -ATGCTAGCCGTTGTGCCGGCTCGCCGGTCGGTCC- 3' (SEQ ID No. 45) and plasmid pBAM25 (published pBK25 by Best, D J et al. Eur J Biochem (1992) 204: 39-49) as template. The PCR product was treated with T4 polynuclectide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with *Sma*I and then treated with alkaline phosphatase.

The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pJLK50 was identified by its restriction pattern and DNA sequencing.

(Paragraph at page 70, line 11 through page 71, line 3.) The approximately 1.8 kbp DNA segment of the monensin PKS gene cluster of Streptomyces cinnamonensis encoding part of the ACP of the loading module 1 and KS of module 1 was amplified by PCR using as primers the synthetic oligonucleotides: 5' - CGTTCCTGAGGTCGCTGGCCCAGGCGTA- 3' (SEQ ID No. 46) and 5' - CGAAGCTTGACACCGCGGCGCGGCGCGG- 5' (SEQ ID No. 47) and a cosmid containing the 5' end of the monensin PKS genes from *S. cinnamonensis* or alternatively chromosomal DNA of *S. cinnamonensis* as template. The PCR product was treated with T4 polynucleotide kinase and then ligated with plasmid pUC18, which had been linearised by digestion with *Sma*I and then treated with alkaline phosphatase. The ligation mixture was used to transform electrocompetent *E.coli* DH10B cells and individual colonies were checked for their plasmid content. The desired plasmid pPFL45 was identified by its restriction pattern.

(Paragraph at page 71, line 21 through page 72, line 2.) For the PCR amplification for plasmid pMO09, the following synthetic oligonucleotides were used as mutagenic primers, one containing a MunI site and the other a PstI site: 5' -

B13 GCGCGCCAATTGCGTGCACATCTCGAT- 3' (SEQ ID No. 48) and 5' - CCTGCAGGCCATCGCGACGACCGCGACCGGTTCGCCG- 3' (SEQ ID No. 49).

(Paragraph at page 72, lines 3-7.) For the PCR amplification for plasmid pM010, the following synthetic oligonucleotides were used as mutagenic primers, one containing B14 a HindIII site and the other an EcoRV site: 5' - GTCTCAAGCTTCGGCATCAGCGGCACCAA- 3' (SEQ ID No. 50) and 5' - CGTGCGATATCCCTGCTCGGCGAGCGCA- 3' (SEQ ID No. 51).

(Paragraph at page 72, line 9 through page 73, line 3.) For the PCR amplification for plasmid pMO13, the following synthetic oligonucleotides were used as mutagenic primers, one containing a PstI site and the other a HindIII site: 5' - GATGGCCTGCAGGCTGCCCGGCGGTGTGAGCA- 3' (SEQ ID No. 52) and 5' - GCCGAAGCTTGAGACCCCCGCCCGGCGCGGTCGC- 3' (SEQ ID No. 53) PCR was carried out on pNTEP2 (GB97/01810) as template using Pwo DNA polymerase and one cycle of: 96°C (1min); annealing at 50°C (3min); and extension at 72°C (1min), and 25 cycles of: 96°C (1min); annealing at 50°C (1min); and extension at 72°C (1min) in the presence of 10% (vol/vol) dimethylsulphoxide. The products were end-repaired and cloned into pUC18 digested with *Sma*I and the ligation mixture was transformed into *E.coli* DH 10B. Plasmid DNA was prepared from individual colonies. The desired plasmids for pMO09 (3.8kbp), pMO10 (3.9 kbp) and pMO13 (4.3 kpb) were identified by their restriction pattern and DNA sequencing.